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RESEARCH ARTICLE

EFFECTS OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACT ON RENAL AND HEPATIC INDICES OF ALBINO WISTAR RATS

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ABSTRACT: Background: Andrographis paniculata, commonly used in herbal medicine, is known for its hepatoprotective and anti-inflammatory properties. However, limited studies have examined its potential effects on renal and hepatic functions. This study aimed to evaluate the impact of A. paniculata ethanolic leaf extract on renal and hepatic indices in male albino rats. **Methods**: Twenty-one male rats were divided into three groups: a control group (distilled water), a low-dose group (10 mg/kg), and a high-dose group (200 mg/kg). The extract was administered orally for 14 days. Serum levels of renal (urea, creatinine, electrolytes) markers were analyzed using ST-100B Electrolyte Analyzer (for electrolytes), Jaffe-slot alkaline picrate method (for creatinine), Berthelot method (for urea), and hepatic (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) markers were analyzed using enzymatic methods. Kidney histopathology was assessed using hematoxylin and eosin staining technique. **Results:** There was a significant increase in AST ($p=0.026$) and ALT ($p=0.003$) levels in the treatment groups, indicating hepatocellular damage. Serum potassium levels also increased significantly ($p=0.0419$), while other renal markers (urea, creatinine, sodium, bicarbonate, chloride, calcium, pH) remained unchanged ($p>0.05$). Histopathological assessment revealed dose-dependent nephrotoxicity, with the high-dose group exhibiting glomerular vacuolations, tubular degeneration, and expanded urinary spaces. **Conclusions:** High-dose A. paniculata administration was associated with hepatocellular damage and potential nephrotoxicity, despite no significant changes in serum renal markers. These findings highlight the need for caution in its therapeutic use and further studies to determine safe dosage thresholds.

Keywords: Andrographis paniculata, Electrolytes, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, renal

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INTRODUCTION:

Natural products have long been used in traditional medicine to treat a variety of diseases due to their bioactive components and therapeutic potentials. Among such therapeutic herbs, *Andrographis paniculata*, widely known as "King of Bitters," has gotten substantial attention in recent years.[1] This plant, which is widely distributed in Asia, has long been used in Ayurveda, Traditional Chinese Medicine, and other herbal medicinal practices due to its numerous pharmacological properties, which include anti-inflammatory, hepatoprotective, and antioxidant effects.[2-4] Diterpenes, lactones, flavonoids, alkenes, ketones, aldehydes, and other active chemicals are abundant in A. paniculata's phytochemical profile.[5-7] The main component of the leaves is andrographolide, sometimes referred to as kalmegin, dioxyandrographolide, neoandrographolide, and dihydroandrographolide.[6,8] This compound is responsible for the bitter taste of the leaf, and enhances its therapeutic properties.[9] Andrographolide, has been reported to demonstrate antioxidant activity, by reducing the level of malondialdehyde (a marker of oxidative stress) and increasing the activities of antioxidant enzymes (such as superoxide dismutase and catalase) in kidney tissues,[10-11] which is crucial in preventing oxidative stress that may lead to renal injury because the kidneys are highly vulnerable to oxidative damage due to their high blood flow and filtration activities.[12-13]

The kidneys and liver are vital organs involved in the body's detoxification, metabolism, and homeostasis. Their functionality is critical for maintaining overall health, as they play pivotal roles in filtering toxins, metabolizing drugs, and regulating biochemical processes. [14-15] However, these organs are highly susceptible to damage from oxidative stress, exposure to toxic substances, and certain pharmaceutical agents. As a result, there is growing interest in exploring natural interventions that could mitigate organ damage and improve their functional integrity.

Studies investigating the pharmacological properties of *A. paniculata* have highlighted its potential in protecting organs from damage induced by oxidative stress and toxic insults. While its hepatoprotective effects have been widely studied, limited research has focused on its effects on both kidney and liver functions in experimental animal models.[11] Understanding these effects is crucial, particularly given the increasing use of *A. paniculata* in herbal formulations for treating a variety of conditions.

This study aims to evaluate the effects of *Andrographis paniculata* leaf extract on the kidney and liver functions of albino rats. By examining biochemical markers and histopathological changes, this research seeks to provide a comprehensive understanding of the plant's impact on these vital organs. The findings could offer valuable insights into its therapeutic potential and safety profile, contributing to the development of evidence-based herbal remedies.

MATERIALS AND METHODS:

Plant materials and extract preparation

The fresh leaves of *A. paniculata* were obtained from Mile 3 market, Diobu, Port Harcourt, Rivers State, Nigeria. The plant identification and authentication were carried out at the Herbarium, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria, by Dr. M. G. Ajuru and a specimen was deposited in the herbarium with voucher number RSUPb0178. The leaves were air-dried for two weeks at room temperature to remove moisture, and grinded into powder. One thousand grams of the powdered A. *paniculata* leaves was soaked in 2000 ml of ethanol and shaken for 72 h; upon completion, the excess solvent was evaporated using a rotary evaporator (Heidolph MX07R−20, PolyScience, USA), to a constant weight and dryness. Prior to administration, the extract was dissolved in appropriate amount of distilled water, such that

10mg/kg was administered to the low dose group, and 200mg/kg to the high dose group respectively.

Laboratory Animals

Twenty-one male Albino Wistar rats with an average weight of 200 g were obtained from the experimental animal unit of the Department of Plant and Animal Biology, Rivers State University, Port Harcourt, Nigeria. All rats were kept in cages in a room temperature maintained at 26–29 °C with a 12-h light–dark cycle for 2 weeks to acclimatize and were allowed free access to food and water ad libitum.

Experimental design

The rats were housed in compartmentalized cage and allowed to acclimatize for two weeks, after which they were divided into three groups of seven animals per group.

Group 1 (control rats): received distilled water and normal feed only, serving as control.

Group 2 rats received a low dose of *A. paniculata* leaf ethanolic extract at 10mg/kg body weight orally for 14 days.

Group 3 rats received high dose of *A. paniculata* leaf ethanolic extract at 200mg/kg body weight orally for 14 days.

The rats were anaesthetized with chloroform and blood samples collected via cardiac puncture on the day 15 of experiment after an overnight fast. Blood samples were taken from the rats and stored for biochemical analysis.

Determination of Biochemical Parameters in Blood Serum

The Concentrations of electrolytes (potassium, sodium, bicarbonate, chloride, total calcium, ionic calcium, pH) were determined using ST-100B Electrolyte Analyzer according to the manufacturer's instructions. Urea was analyzed using Berthelot method as described by Akpotaire & Seriki, (2023).[12] Creatinine was determined using Jaffe-slot alkaline picrate method according to Akpotaire & Seriki, (2023).[12] ALT, AST and ALP were determined using enzymatic methods as described by Kim *et al.,* (2020) and Wang *et al.,* (2020).[13-14]

AST is released into the bloodstream when liver cells are damaged, making it useful for detecting liver injury. ALT is primarily found in liver cells. When liver cells are damaged or inflamed, ALT is released into the bloodstream, making it a more sensitive indicator of liver injury. ALP is an enzyme found in the liver, bones, kidneys, and bile ducts. It is particularly useful for detecting bile duct obstructions or cholestasis (impaired bile flow). Hence, it is typically used in conjunction with other liver tests to determine the cause of elevation. Creatinine and urea measurement both reflect glomerular filtration rate (GFR), the electrolytes are markers of renal tubular function; these parameters define kidney function for the clinician.

Histological study

The kidney was harvested from the sacrificed rats and immediately fixed in 10% formal saline for histological studies using the H &E staining technique.

STATISTICS

The results of the study were analyzed using GraphPad Prism Version 8.0.2 (263), expressed as Mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was carried out, with 95% confidence interval and p-value < 0.05 was considered statistically significant.

RESULTS:

Table 1: The quantitative phytochemical analysis of *Andrographis paniculata* leave extract

Components (mg/g)	Mean ± SD
Tannins	0.20 ± 0.01
Flavonoids	4.70 ± 0.01
Cyanogenic Glycoside	2.55 ± 0.02
Phenol	11.77 ± 0.01
Reducing Sugar	5.67 ±0.01
Alkaloids	1.76 ± 0.01
Saponins	1.3 ± 0.02

Table 2: Levels of Urea and Creatinine in group 1, 2 and 3 after 14 days of treatment compared with control group

	Group 1 (Control) n = 7	Group 2 (Low Dose) n = 7	Group 3 (High Dose) n =7	F-Value	p-Value
Urea (mmol/L)	2.73 ± 1.10	2.27 ± 0.49	2.60 ± 0.50	0.3049	0.7454
Creatinine (µmol/L)	62.50 ± 6.61	60.33 ± 3.06	62.25 ± 1.71	0.2328	0.7975

Table 3: Levels of Electrolytes in group 1, 2 and 3 after 14 days of treatment compared with control group

	Na (mmol/L)	K (mmol/L)	HC03 (mmol/L)	Cl (mmol/L)	pH	TCa (mmol/L)	Ica (mmol/L)	NCa (mmol/L)
Group 1	126.3 ± 7.89	3.53 ± 0.31	22.25 ± 1.50	105.5 ± 2.52	7.48 ± 0.15	1.64 ± 0.54	3.09 ± 4.56	0.89 ± 0.09
Group 2	135.70 ± 2.89	4.10 ± 0.53	19.00 ± 6.25	107.0 ± 1.00	7.37 ± 0.06	1.09 ± 0.74	0.64 ± 0.24	0.67 ± 0.26
Group 3	134.80 ± 3.86	4.38 ± 0.36	18.00 ± 3.27	107.80 ± 5.50	7.38± 0.09	1.76 ± 0.66	0.89 ± 0.35	0.92 ± 0.31
F-Value	3.268	4.839	1.332	0.3734	1.218	1.018	0.8677	1.065
p-Value	**0.0918**	**0.0419**	**0.3167**	**0.6998**	**0.34544**	**0.4039**	**0.4560**	**0.3889**

Table 4: Activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) in group 1, 2 and 3 after 14 days of treatment compared with control group

	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 1 (Control) n=7	5.00 ±1.73	4.00 ± 0.01	36.33 ± 1.53
Group 2 (low Dose) n=7	18.50 ± 3.32	14.00 ± 4.24	63.75 ± 2.22
Group 3 (High Dose) n=7	24.00 ± 11.46	9.50 ± 1.00	48.50 ± 22.04
F-Value	5.881	12.04	3.576
p-Value	**0.026**	**0.003**	**0.077**

Histology Result

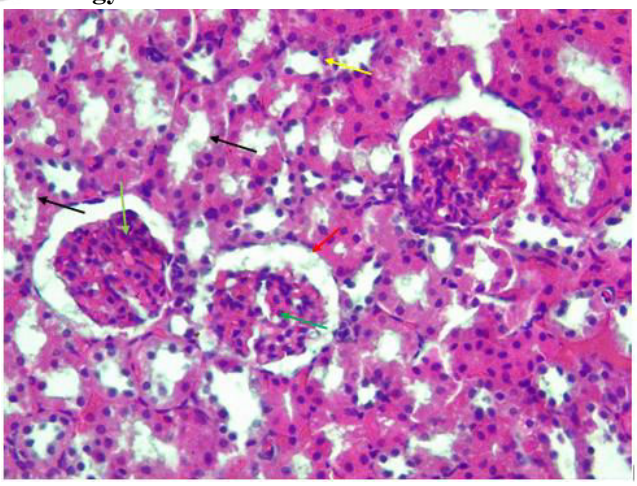

Figure 1: Photomicrograph of a section of a normal kidney tissue taken from control group rats (Control) showing glomerulus (green arrows), urinary space (red arrows), proximal tubules (yellow arrow) and distal tubules (black arrow), (H&E x100 (Leica DM 750, Camera ICC50 E x400).

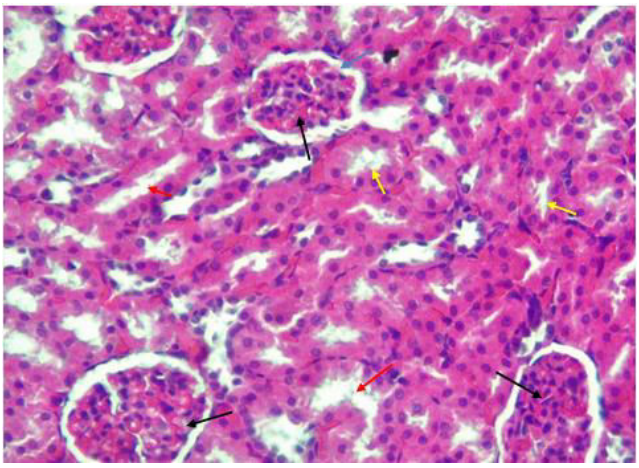

Figure 2: Photomicrograph of a section of a normal kidney tissue (black arrows) from Wistar rats of group treated with low dose of *A. paniculata*. Section displayed glomerulus (black arrows), urinary space (blue arrows), proximal tubules (yellow arrow) and distal tubules (red arrow), (H&E x400 (Leica DM 750, Camera ICC50 E x400).

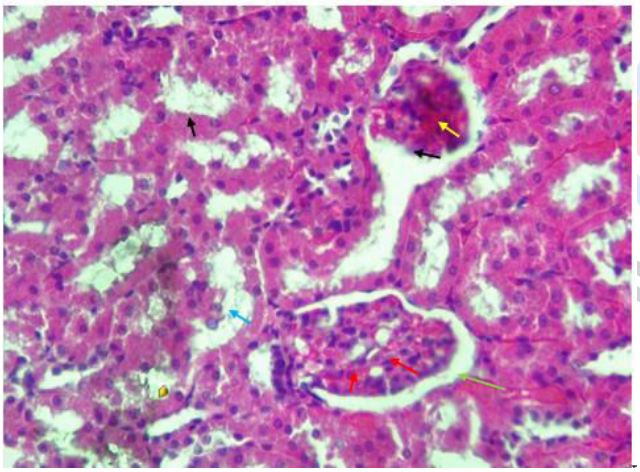

Figure 3: Photomicrograph of a section of kidney tissue taken from Wistar rats treated with High Dose *A. paniculata*. Section showed glomerular tissues vacuolations (red arrows), degeneration (Yellow arrows) with cellular and tissue damages. The tubules luminal spaces are enlarged in proximal (black arrows) and distal tubules (blue arrows) and urinary space expansion of the bowman's capsule (green arrows) (H&E x100).

DISCUSSION:

Ingestion of *A. paniculata* ethanolic leaf extract may be harmful to vital organs such as the kidney and the liver if the dose is not closely regulated. The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes of acute hepatocellular damage namely ALT, AST and ALP as a marker for hepatobiliary damage. However, of these marker enzymes, ALT is the most reliable.[19]

A. paniculata ethanolic leaf extract was found to be associated with increased AST and ALT activities which is indicative of hepatotoxic effects. This finding agrees with the findings of Kaewdech *et al.*(2022)[20] and Owoade *et al.,* (2022),[21] but disagrees with the report of Nasir *et al.,* (2013)[22] whose study demonstrated that *A. paniculata* possesses significant hepatoprotective effects against Carbon Tetrachloride – Induced hepatotoxicity in rats. Liver function is mostly evaluated using AST, ALT, and ALP assays. An increase in the activities of the hepatic enzymes in the plasma is an indication of liver dysfunction/ abnormalities.[23] This study showed increase in the activities of AST and ALT of the treated rats compared with the untreated (control) rats. Significantly high aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with normal alkaline phosphatase (ALP) typically suggests hepatocellular damage rather than a cholestatic process.

There was significantly elevated Potassium level among *A. paniculata* treated rats compared with the untreated rat group. Other kidney function markers analyzed, including urea, creatinine and the electrolytes sodium, chloride, bicarbonate, as well as other commonly associated parameters such as ionized calcium, total calcium, non-ionized calcium and pH showed no significant differences in the low dose and high dose groups, compared to the control group.

A significantly high potassium level (hyperkalemia) is often associated with impaired kidney function and other systemic conditions. The kidneys play a central role in maintaining potassium balance by excreting excess potassium through urine. Hyperkalemia often indicates that the kidneys are not filtering and excreting potassium efficiently, which may occur in

acute kidney injury.[24-25] The routine assessment of accurate kidney function which is a prerequisite for well-informed clinical decision making in several areas involves several parameters including the glomerular filtration rate (GFR), urea, creatinine, and electrolytes.[26]Normal urea and creatinine levels with high potassium levels can indicate several potential conditions, as urea and creatinine are primary markers of glomerular function, while potassium levels are closely linked to renal excretion of electrolytes. Even though urea and creatinine levels were not significantly affected the kidneys may still be struggling with their ability to excrete potassium properly. Elevated potassium (hyperkalemia) can occur before kidney function is sufficiently impaired to cause noticeable changes in urea and creatinine levels. In addition, certain medications, such as ACE inhibitors, angiotensin receptor blockers (ARBs), potassium-sparing diuretics, or NSAIDs, can cause an increase in potassium levels. [27-28] Therefore there may be need to understand the mechanism of action of *A. paniculata.*

However, the histological examination of the kidney tissue revealed glomerular tissues vacuolations, degeneration, with cellular and tissue damages, luminal space enlargement in proximal and distal tubules, and urinary space expansion of the bowman's capsule in the high dose treatment group. While the low dose treatment group showed normal kidney histology. This may signify a dose-dependent nephrotoxicity associated with *A. paniculata* administration. The nephrotoxic effect of *A. paniculata* on the kidney, as indicated by hyperkalemia correlates with the observed histological damage (renal dysfunction).

The finding of glomerular tissues vacuolations, degeneration, with cellular and tissue damages are concurrent with the reports of Phetruen *et al.,* (2023)[1] who observed that andrographolide, a bioactive component found in *A. paniculata*, induced cytotoxicity, and affected various cellular processes, including vacuole fragmentation, endoplasmic reticulum stress, lipid droplet accumulation, reactive oxygen species levels, and compromised cell integrity in *Saccharomyces cerevisiae*.

The quantitative phytochemical analysis of *A. paniculata* leave ethanolic extract were indicative of phenol, reducing sugar, flavonoids, cyanogenic glycoside and alkaloids. Saponins and tannins were present in trace amounts. Phenol had the highest amount compared to other phytochemical components. This is in concurrence with the report of Nagajothi *et al.,* (2018),[29] who screened aqueous and ethanolic extracts of *Andrographis paniculata* leave for fourteen phytochemicals. The presence of these phytochemical components are the possible reasons behind the wide use and inclusion of *A. paniculata* in herbal remedies. [3,30-31]

CONCLUSION:

This study investigated the effects of *Andrographis paniculata* ethanolic leaf extract on kidney and liver functions in albino Wistar rats. The findings highlight that while the low dose treatment group exhibited no significant biochemical alterations or histological abnormalities, the high dose group demonstrated notable kidney tissue damage, including glomerular vacuolations, tubular degeneration, and expanded urinary spaces. Although there were no significant changes in serum markers such as urea, creatinine, or major electrolytes at either dose, elevated potassium levels in the treatment groups and histological evidence of nephrotoxicity at high doses suggest a dose-dependent impact on renal integrity. Further more, medications, such as ACE inhibitors, angiotensin receptor blockers (ARBs), or potassium-sparing diuretics, can cause an increase in potassium levels. Therefore, there is need for research into understanding the mechanism of action of *A. paniculata*, This will help to explain whether it falls under ACE inhibitors, ARBs or potassium-sparing diuretics as the case may be. Additionally, the significant increases in AST and ALT levels indicate

potential hepatocellular damage associated with *A. paniculata* administration.

These results underscore the need for caution in the therapeutic use of *A. paniculata*, particularly at higher doses, as it may compromise liver and kidney function. The study also highlights the importance of further research to elucidate the underlying mechanisms of these toxic effects and to establish safe dosage thresholds. Therefore, there is need to elucidate the underlying mechanisms of these hepatotoxic and nephrotoxic effects through gene expression studies and evaluation of oxidative stress markers.

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